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Mutagenicity of the Fractionated Organic Emissions from Diesel, Cigarette Smoke Condensate, Coke Oven, and Roofing Tar in the Ames Assay

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16. ABSTRACT

Mobile and stationary sources emit particle-bound organics that have demonstrated mutagenicity. The objective of this study was to measure the mutagenicity of the fractionated organic emissions from diesel, cigarette smoke condensate (CSC), coke oven and roofing tar in the Ames assay. This study demonstrated significant biological differences among the four emission sources. Within each source, the relative mutagenicity of each fraction was significantly different in the presence and absence of an exogenous metabolic activation. In the diesel sample, over 90% of the mutagenic activity is located in the aromatic and polar neutral (PN) fractions, and a significant portion of this activity can be accounted for by nitro-PNAs. Most of the mutagenicity of the coke oven main sample was found in the BASE and PN fractions which contained aromatic amines and nitrogen heterocycles. The CSC sample also had a high percent of the mutagenic activity in the BASE fraction. Chemical analysis however, indicates that the components in the CSC differed significantly from those of the coke oven main sample. The roofing tar sample contained aromatic and polar mutagenic constituents that were not NO_2-PNAs. Although the specific mutagens in these different sources are not identical, they all cause frameshift mutations and appear to be compounds that could be classified as polycyclic organic matter (POM).

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Mutagenicity of the Fractionated Organic Emissions From Diesel, Cigarette Smoke Condensate, Coke Oven, and Roofing Tar in the Ames Assay

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Mobile and stationary combustion sources emit particle-bound organics that, after extraction, are mutagenic to *Salmonella typhimurium*. In this study, the organic emissions from diesel, cigarette smoke condensate (CSC), coke oven, and roofing tar were fractionated and compared for mutagenicity in the Ames assay. This study demonstrated major differences in the distribution of mutagenicity among the four emission sources. Within each source, the relative mutagenicity of each fraction was significantly different in the presence and absence of an exogenous metabolic activation. In the diesel sample, over 90% of the mutagenic activity is located in the aromatic and polar neutral (PN) fractions; nitrated polynuclear aromatic hydrocarbons (NO_2-PNAs) can account for a significant portion of this activity. Most of the mutagenicity of the coke oven main sample was found in the organic base (BASE) and PN fractions, which contained aromatic amines and nitrogen heterocycles. The CSC sample also had a high percentage of the mutagenic activity in the BASE fraction. Chemical analysis, however, indicates that the components in the CSC differed from those of the coke oven main sample. The roofing tar sample, which was not mutagenic in the absence of metabolic activation, contained several components that were very mutagenic after fractionation. This may be due to the separation of toxic components from the mutagenic components. The roofing tar emissions contained aromatic and polar mutagenic constituents. Although the specific mutagens in these different sources are not identical, they all cause frameshift mutations and appear to be compounds that could be classified as polycyclic organic matter.

Key words: mutagenicity, fractionation, diesel, cigarette smoke condensate, coke oven, roofing tar

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INTRODUCTION

Organic emissions from several sources, including combustion products such as chimney soot, coal tars, and cigarette smoke, are recognized as human carcinogens. The carcinogenic constituents historically identified in coal tars and soot extracts are primarily the polynuclear aromatic hydrocarbons (PNAs) such as benzo(a)pyrene [B(a)P] [Cook and Kennaway, 1938]. When the Ames *Salmonella typhimurium* mutagenicity bioassay [Ames et al, 1975] was applied to the fractionated combustion emissions from cigarette smoke condensates (CSC) [Hutton and Hackney, 1975; DeMarini, 1981] and diesel particles [Huisingh et al, 1978], most of the mutagenic activity was not found in the aromatic (fraction that contained PNAs) but in the basic fraction for the CSC and the more polar neutral fractions for the diesel. Recent advances in both chemical analytical techniques and bioassay methods have led to the identification of several classes of mutagenic and carcinogenic constituents, including aromatic amines, substituted PNAs (eg, nitrated-PNAs), and heterocycles. Chemical fractionation and characterization methods applied to the characterization of mutagens in one source (eg, diesel emissions) have differed from the chemical fractionation techniques used for another source (eg, CSC), therefore making it difficult to directly compare two different sources.

In order to characterize and assess the mutagenicity and carcinogenicity of organics associated with diesel particulate emissions, a major study was undertaken by the U.S. Environmental Protection Agency to compare the extractable organics from diesel particles to organic emissions from three sources that are recognized as human carcinogens, including coke ovens, roofing tar, and cigarette smoke [Lewtas et al, 1981]. These organic emissions have also been assayed in a battery of in vitro and in vivo mutagenesis and carcinogenesis bioassays [Lewtas, 1983; Nesnow et al, 1982, 1983], and the data has been used in the development of methodology for comparative cancer risk assessment [Albert et al, 1983; Lewtas et al, 1983]. The organic emissions from these four sources were also fractionated by chemical class and bioassayed for mutagenicity. This paper presents the comparative mutagenicity of the fractionated emissions in the Ames *Salmonella typhimurium* plate incorporation assay. The detailed chemical characterization of each fraction is reported elsewhere (Williams et al, unpublished results). The purpose of this paper is to compare the mutagenicity of each fraction and the distribution of mutagenicity between fractions for each emission sample. The chemical constituents found in those fractions that account for most of the mutagenicity are discussed in order to clarify the similarities and differences observed.

MATERIALS AND METHODS

Sample Collection

Diesel automotive emissions. The diesel particulate emissions were collected from a light-duty diesel passenger car (Oldsmobile 350) by dilution tunnel sampling procedures described by Lewtas et al [1981].

Cigarette smoke condensate. The CSC was generated and collected by the method of Patel [1977] as described by Lewtas et al [1981].

Coke oven emissions. The coke oven main sample was collected from a separator between the gas collector main and the primary coolers within the coke oven battery located at Republic Steel in Gadsden, Alabama, approximately 60 mi northeast of Birmingham. The coke oven main sample represented a sample of the vapors and other by-products normally recovered in the by-product gas collector during the "charging" operation of the coking cycle [Albert et al, 1983].

Roofing tar emissions. Roofing tar emissions were generated and collected by the methods described in Lewtas et al [1981]. The tar utilized in this study was derived from coal and has been incorrectly reported elsewhere as an asphalt [Claxton and Huisingh, 1980] and pine pitch [Nesnow et al, 1982] tar.

Extraction

The particulate emissions collected from the roofing tar pot and diesel vehicle were Soxhlet-extracted with dichloromethane (DCM), as described by Lewtas et al [1981], to remove the organics. The coke oven mains were totally soluble in DCM and therefore were not extracted. The CSC was removed from the condenser by rinsing with a minimal amount of acetone, and the acetone was removed from the sample by rotary evaporation. The solvents used to extract or condense the organics from each sample were removed by evaporation under nitrogen, and the dried extracts were stored at or below −80°C in the dark.

Chemical Fractionation

Approximately 1 g of each extract was dissolved in DCM, passed through a 0.5 μm Teflon filter, and fractionated by liquid/liquid partitioning as described by Kolber et al [1980]. This partition scheme (Fig. 1) separates the sample into organic acids (ACID), organic bases (BASE), cyclohexane insolubles (CI), polar neutrals (PN), non-polar neutrals (NPN), and polynuclear aromatic hydrocarbons (PNA).

The PNA fraction was further separated by a preparative scale adsorption chromatographic method (Williams et al, unpublished results) summarized here. Milligram quantities of the PNA fraction were separated on a low pressure Lobar® silica gel B column (E. Merck, Darmstadt, West Germany) operated with a Perkin Elmer (Norwalk, CT) series 1 pump at 29.9 ml/min with a pulse dampener (FMI, Oyster Bay, NY) and an ultraviolet (UV) 254-nm in-line detector (Gilson, Middleton, WI).

The unsubstituted PNAs were eluted from the column with 2% DCM in hexane. This fraction was designated as PNA1. Standard solutions of phenanthrene and coronene were eluted in this fraction. More polar material (eg, substituted PNAs) was eluted in subsequent fractions using progressively more polar solvents: fraction PNA2 was eluted with 50% DCM in hexane, PNA3 with 100% DCM, and PNA4 with 10% methanol in DCM.

The PNA1, NPN, and CI liquid/liquid partition fractions were analyzed by high pressure liquid chromatography (HPLC) to evaluate the separation of fluorescent (313-nm excitation, 425-nm emission) unsubstituted aromatic compounds (254-nm absorbing). This HPLC analysis confirmed that the unsubstituted PNAs (eg, anthracene, phenanthrene, B(a)P, perylene, etc) were contained within the PNA1 fraction with very little spillover into the other fractions. Minor amounts of PNAs were present in the CI and NPN fractions in the coke oven and roofing tar samples (Williams et al, unpublished results).

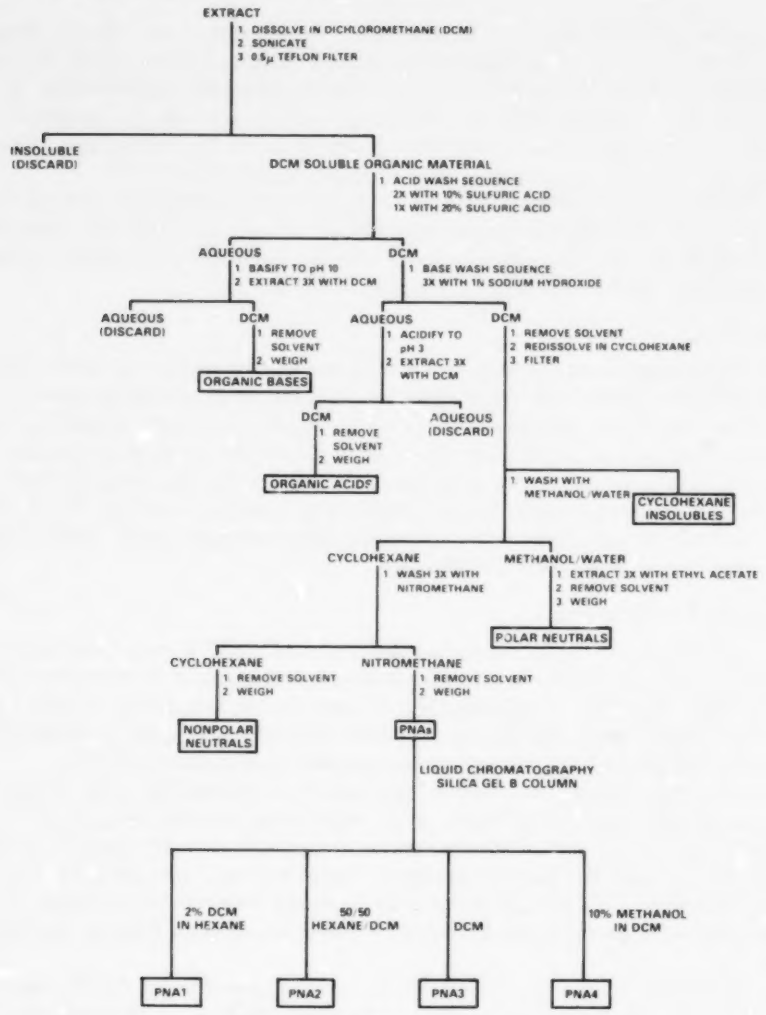

Fig. 1. Fractionation scheme.

After partitioning, solvent was removed from each fraction using rotary evaporation (reduced pressure at 35°C). Aliquots of each fraction were sequentially taken to dryness under a stream of dry nitrogen gas in tared 1-dram glass vials. To ensure total solvent removal, each fraction was dried to a constant mass.

Mutagenesis Method

Ames *Salmonella typhimurium* plate incorporation test. *Salmonella typhimurium* strain TA98 (supplied by Dr. Bruce Ames) was used to detect reverse mutations from histidine dependence to histidine independence in the Ames plate incorporation assay [Ames et al, 1975] with the following minor modifications

[Claxton, 1981]: a trace amount of histidine was incorporated into the bottom minimal nutrient medium, and the plates were incubated for 72 hr. Plates were scored electronically with an Artek Model 880 Automatic Colony Counter. Each sample was dissolved in dimethylsulfoxide (DMSO) and assayed at a minimum of five doses in triplicate plates with and without metabolic activation (S9), when sufficient sample was available, and below the concentration where toxicity could be observed by the clearing of the background lawn. The bioassay included positive and negative (solvent) controls. The positive controls used for strain TA98 were 2-nitrofluorene (2-NF), 3 μg/plate, without activation (−S9), and 2-anthramine (2-AA), 0.5 μg/plate, with activation (+S9). The S9 mixture (1.04 mg protein/plate) was tested for sterility before and after the assay was performed. This S9 concentration was determined to be optimal for the unfractionated (NEAT) samples [Williams and Lewtas, 1985].

Data analysis. The mutagenicity data was analyzed using a non-linear model [Stead et al, 1981], and the Ames Test Curve Fitting Program [Hasselblad et al, 1980] to determine the slope of the dose-response curve (model slope). These values are referred to as specific mutagenic activity (rev/μg). The weighted mutagenic activity of each fraction relative to the total extract is obtained by multiplying the specific mutagenic activity (rev/μg) by the percentage of the total mass recovered in each fraction. The percent of total mutagenic activity in each fraction is determined from the weighted mutagenicity of that fraction ($\times 100$) divided by the total of the weighted mutagenicities of all the fractions. This approach assumes that any loss in mutagenicity through the chemical fractionation is equally distributed across all fractions.

RESULTS

Mutagenicity of the Unfractionated Extract

The mutagenicity of the unfractionated (NEAT) extracts from the four environmental samples in *S typhimurium* TA98 are shown in Table I both with and without S9 activation. The mutagenicity of these unfractionated emissions in TA1535, TA1537, TA1538, and TA100 has been reported elsewhere [Claxton and Huisingh, 1980; Claxton, 1981]. The diesel NEAT extract was the only NEAT sample positive without S9; addition of S9 decreased the mutagenicity of this sample by 67%. All four NEAT extracts were mutagenic in the presence of S9. The effect of S9 concentration, as reported by Williams and Lewtas [1985], was optimal at the concentration reported here. Although the NEAT extract from the roofing tar sample appeared to be mutagenic at the lowest doses (the most potent in rev/μg), this extract was so highly toxic that it was not possible to observe a doubling of the spontaneous revertants. The large confidence limits (0.5–78.1) on the model slope for the roofing tar sample also indicate that the slope determined by the non-linear model is only approximated (6 rev/μg) (Table VI). The slope values are used to calculate the percent recovery of mutagenicity by comparing the sum of the fractions to the NEAT extracts for each source (Tables III–VI).

Mutagenicity of Each Fraction

The mutagenicity of each of the organic fractions in *S typhimurium* TA98 is shown in Table IIA–C both with and without the S9 metabolic activation system. In order to compare directly fractions across the four emission sources, the same fraction from each of the four sources was bioassayed simultaneously except where noted. In

TABLE I. Mutagenicity of Unfractionated (NEAT) Extract From Four Environmental Samples Using *Salmonella typhimurium* Strain TA98*

Sample and controls	Dose (μg)	NEAT Extract rev/plate mean ± SD	
		−S9	+S9
2-NF	3.0	277 ± 6.4	
2-AA	0.5		490 ± 39.6
Diesel	0	23 ± 0.7	46 ± 10.6
	60	140 ± 20.7	76 ± 13.1
	100	220 ± 15.9	150 ± 4.5
	200	452 ± 46.5	324 ± 8.1
2-NF	3.0	308 ± 11.7	
2-AA	0.5		1,609 ± 73.3
CSC	0	24 ± 5.5	44 ± 1.7
	30	25 ± 9.6	55 ± 7.2
	50	30 ± 6.4	69 ± 6.7
	100	28 ± 1.2	100 ± 2.9
	300	24 ± 4.0	103 ± 18.2
	500	26 ± 6.6	85 ± 4.6
2-NF	3.0	290 ± 26.6	
2-AA	0.5		711 ± 11.5
Coke oven	0	33 ± 8.6	43 ± 8.4
	10	28 ± 0.6	196 ± 28.0
	20	31 ± 9.1	322 ± 18.7
	30	36 ± 4.6	412 ± 22.1
	60	39 ± 0.0	621 ± 21.7
	100	40 ± 9.1	737 ± 43.7
	200	43 ± 5.6	756 ± 70.8
	600	36 ± 2.5	939 ± 59.4
2-NF	3.0	308 ± 11.7	
2-AA	0.5		1,609 ± 73.3
Roofing tar	0	24 ± 5.5	44 ± 1.7
	3	26 ± 5.8	40 ± 0.0
	5	33 ± 9.6	42 ± 1.5
	10	26 ± 6.9	52 ± 5.9
	30	23 ± 4.4	65 ± 3.8
	50	17 ± 1.0	60 ± 10.7

*When possible, these values represent a mean of triplicate plates.

one case (PNA2, Table IIB), the lowest dose tested resulted in very high plate counts. Insufficient quantities of PNA1–4 fractions were available to repeat these assays, and the statistical parameters suggested that the model slopes were acceptable.

Distribution of Mutagenic Activity in Each Source

The distribution of the mutagenic activity between fractions within each source is shown in Tables III–VI. The sum of the weighted mutagenicities for each fraction (Total fractionated) was compared to the mutagenicity of the NEAT sample to calculate the recovery of mutagenic activity. Recovery of more mutagenic activity after fractionation (>100%) was observed for the diesel (Table III) and coke oven samples (Table V) in the presence of S9. Recovery of substantially less (27.3%) mutagenicity after fractionation was observed for the CSC. This appears to be directly related to the poor recovery of mass after fractionation by solvent partitioning

(47.6%). The low mass recovery of the CSC sample was not due to emulsion or precipitation problems and could in part be due to the low efficiency of the ACID/BASE extraction (Williams et al, unpublished results). The results for all four samples are described below.

Diesel automotive emissions. Although the NPN fraction contained the greatest percent total mass (75%) after fractionation, this fraction had less than 2% of the mutagenic activity (Table III). The PN fraction contained 45% and 52% of the mutagenic activity in the absence and presence of S9, respectively. The other highly polar neutral fraction, the CI fraction, contained 32% and 16% of the mutagenic activity in the absence and presence of S9, respectively. Together these two fractions account for 77% and 68% of the mutagenicity. The data indicate that direct-acting mutagens were present in many of the diesel fractions. The PNA2 and PNA3 fractions also made a major contribution to the total mutagenicity. These two fractions accounted for 21% of the mutagenicity in the presence of S9 and 8% in the absence of S9.

Cigarette smoke condensate. The polar CI fraction contained the greatest percent of the total mass (74%) and contributed 25% of the mutagenic activity in the presence of S9 (Table IV). The BASE fraction accounted for the majority of the mutagenic activity in the presence of S9 (66%). Mutagenic activity (0.1–6%) was distributed across all fractions in the presence of S9 except for the PN fraction, which was not mutagenic. Direct-acting mutagens appeared to be present in only the PNA3 and PNA4 fractions. The CSC NEAT sample was negative in the absence of S9 and even after fractionation showed very little mutagenicity in the absence of S9.

Coke oven emissions. The CI fraction contained the greatest percent of the total mass (58%) and in the presence of S9 accounted for 31% of the mutagenic activity (Table V). The BASE fraction accounted for 37% of the mutagenic activity in the presence of S9 and contained only 4% of the mass. The remainder of the mutagenic activity (32%) was distributed across all the remaining fractions in the presence of S9. Very little direct-acting mutagenicity was detected after fractionation. Similar to the CSC, the coke oven direct-acting mutagenicity was distributed between the PNA3 (18%) and PNA4 (82%) fractions.

Roofing tar emissions. The NPN and PNA1 fractions represented the greatest percent of the total mass but contained less than 10% of the mutagenic activity (Table VI). The two polar fractions PN and CI contained most of the mutagenicity in the presence of S9 (17% and 58%, respectively). The remainder of the mutagenicity was distributed across all the other fractions with the PNA1 fraction (containing unsubstituted PNAs), accounting for 5% of the mutagenicity and the PNA2 fraction 8% of the mutagenicity. Although the NEAT roofing tar sample was negative in the absence of S9, after fractionation, the total mutagenicity (sum of the weighted mutagenicity) was nearly as great in the absence of S9 (4.3 rev/μg) as in the presence of S9 (5.3 rev/μg). The CI fraction contained most of the direct-acting mutagenicity (89%).

DISCUSSION

This paper reports for the first time, to the best of our knowledge, the systematic comparison of the mutagenicity of fractionated organic emissions from a diesel automobile (soot), roofing tar (coal tar derived), coke oven, and cigarettes. Historically the PNAs have been identified as the principal carcinogenic constituents of coal

TABLE IIA. Mutagenicity of Organic Fractions From Four Environmental Samples Using *Salmonella typhimurium* Strain TA98: Acid, Base, NPN Fractions*

Sample and controls	Acid Fraction rev/plate mean ± SD			Base Fraction rev/plate mean ± SD			NPN Fraction rev/plate mean ± SD		
	Dose (μg)	−S9	+S9	Dose (μg)	−S9	+S9	Dose (μg)	−S9	+S9
2-NF	3.0	205 ± 9.3		3.0	53 ± 0.0[a]		3.0	233 ± 27.3	
2-AA	0.5		670 ± 10.1	0.5		718 ± 16.1	0.5		454 ± 34.2
Diesel	0	27 ± 8.1	41 ± 9.1	0	24 ± 5.7	53 ± 3.5	0	22 ± 5.3	34 ± 13.9
	20	206 ± 17.2	137 ± 15.0	20	68 ± 0.0	52 ± 8.6	20	28 ± 1.5	43 ± 6.1
	60	473 ± 9.2	407 ± 43.9	60	155 ± 39.7	112 ± 7.2	60	26 ± 6.0	42 ± 0.7
	100	727 ± 35.9	622 ± 33.9	100	248 ± 96.1	207 ± 13.0	100	30 ± 1.7	37 ± 3.5
	200	1,086 ± 66.0	706 ± 11.3	200	685 ± 11.0	482 ± 29.9	200	32 ± 6.1	44 ± 8.5
	600	1,680 ± 80.0	1,025 ± 29.7	600	1,626 ± 5.7	1,549 ± 45.3	600	29 ± 3.8	67 ± 15.5
	1,000	1,552 ± 56.7	1,082 ± 137.9	1,000	—	1,919 ± 0.0	1,000	28 ± 1.7	83 ± 16.8
2-NF	3.0	205 ± 9.3		3.0	110 ± 8.1		3.0	233 ± 27.3	
2-AA	0.5		670 ± 10.1	0.5		718 ± 16.1	0.5		454 ± 34.2
CSC	0	27 ± 8.1	41 ± 9.1	0	21 ± 3.0	53 ± 3.5	0	22 ± 5.3	34 ± 13.9
	10	27 ± 7.9	38 ± 6.8	20	15 ± 1.5	58 ± 5.8	10	30 ± 6.6	40 ± 2.5
	30	29 ± 3.5	56 ± 3.5	60	18 ± 7.5	89 ± 13.3	30	26 ± 5.5	47 ± 4.4
	50	31 ± 6.7	50 ± 1.5	100	25 ± 10.0	109 ± 15.7	50	26 ± 11.0	43 ± 8.1
	100	29 ± 2.5	60 ± 4.5	200	22 ± 7.0	218 ± 30.7	100	33 ± 4.9	53 ± 8.1
	300	41 ± 3.2	87 ± 6.8	600	22 ± 8.5	333 ± 4.7	300	—	93 ± 0.0
	500	—	129 ± 0.0	1,000	22 ± 5.9	350 ± 21.2	500	—	56 ± 0.0
2-NF	3.0	205 ± 9.3		3.0	110 ± 8.1		3.0	233 ± 27.3	
2-AA	0.5		670 ± 10.1	0.5		718 ± 16.1	0.5		454 ± 34.2

(continued)

TABLE IIA. Mutagenicity of Organic Fractions From Four Environmental Samples Using *Salmonella typhimurium* Strain TA98: Acid, Base, NPN Fractions* (Continued)

Sample and controls	Acid Fraction rev/plate mean ± SD			Base Fraction rev/plate mean ± SD			NPN Fraction rev/plate mean ± SD		
	Dose (μg)	−S9	+S9	Dose (μg)	−S9	+S9	Dose (μg)	−S9	+S9
Coke oven	0	27 ± 8.1	41 ± 9.1	0	21 ± 3.0	53 ± 3.5	0	22 ± 5.3	34 ± 13.9
	10	29 ± 8.1	56 ± 4.9	20	30 ± 2.0	1,318 ± 57.0	20	30 ± 2.9	120 ± 9.1
	30	31 ± 4.6	70 ± 8.2	60	32 ± 8.7	2,234 ± 113.9	60	39 ± 5.2	194 ± 15.3
	50	28 ± 7.5	97 ± 6.5	100	28 ± 7.2	2,230 ± 48.0	100	31 ± 12.0	195 ± 9.1
	100	26 ± 2.1	123 ± 8.0	200	29 ± 7.6	2,213 ± 168.3	200	26 ± 5.6	184 ± 32.3
	300	24 ± 0.0	112 ± 0.0	600	20 ± 4.0	1,014 ± 398.2	600	30 ± 0.6	80 ± 5.3
				1,000	22 ± 3.6	33 ± 10.6	1,000	17 ± 1.1	53 ± 14.8
2-NF	3.0	205 ± 9.3		3.0	110 ± 8.1		3.0	233 ± 27.3	
2-AA	0.5		670 ± 10.1	0.5		718 ± 16.1	0.5		454 ± 34.2
Roofing tar	0	27 ± 8.1	41 ± 9.1	0	21 ± 3.0	53 ± 3.5	0	22 ± 5.3	34 ± 13.9
	20	30 ± 3.2	58 ± 7.8	20	22 ± 4.4	259 ± 11.5	20	28 ± 4.7	44 ± 7.1
	60	23 ± 1.7	64 ± 10.2	60	19 ± 0.0	426 ± 25.4	60	27 ± 10.7	66 ± 11.5
	100	25 ± 1.0	68 ± 5.5	100	20 ± 5.6	625 ± 120.6	100	26 ± 2.6	72 ± 17.0
	200	11 ± 5.1	74 ± 21.9	200	18 ± 0.6	559 ± 126.3	200	23 ± 3.8	64 ± 14.1
	600	Toxic	3 ± 1.4	600	10 ± 4.5	47 ± 17.1	600	9 ± 5.5	35 ± 6.8
	1,000	Toxic	Toxic	1,000	Toxic	52 ± 70.0	1,000	9 ± 3.8	19 ± 6.1

*When possible, these values represent a mean of triplicate plates.

[a]The diesel base fraction when repeated gave very similar results and a low value for 2-NF; therefore, we concluded that this control chemical had deteriorated. This fraction contributes a very minor portion of the mutagenicity to the total diesel sample (see text).

tars and soot extracts [Dipple, 1976; Kipling, 1976]. These early findings led to the generalization that PNAs may be the major carcinogenic constituents of other soots (eg, diesel emissions) and coal-derived emissions (eg, coke oven emissions). Separation of PNAs by solvent partitioning does not result in a purified PNA fraction and has been shown to contain direct-acting mutagens [Lewtas et al, 1982]. The PNA fraction of each of the organic emissions reported here was further fractionated on silica gel to obtain a purified fraction of unsubstituted PNAs (PNA1). The PNA1

TABLE IIB. Mutagenicity of Organic Fractions From Four Environmental Samples Using *Salmonella typhimurium* Strain TA98: PNA1, PNA2, PNA3, PNA4 Fractions*

Sample and controls	Dose (μg)	PNA1 Fraction rev/plate mean ± SD −S9	PNA1 Fraction rev/plate mean ± SD +S9	Dose (μg)	PNA2 Fraction rev/plate mean ± SD −S9	PNA2 Fraction rev/plate mean ± SD +S9
2-NF	3.0	204 ± 7.0		3.0	204 ± 7.0	
2-AA	0.5		512 ± 52.2	0.5		512 ± 52.2
Diesel	0	37 ± 8.7	45 ± 10.8	0	37 ± 8.7	45 ± 10.8
	20	19 ± 7.6	52 ± 9.1	20	1,029 ± 9.7	1,201 ± 96.0
	60	24 ± 4.5	67 ± 5.1	60	1,703 ± 50.9	2,314 ± 80.3
	100	26 ± 1.0	76 ± 3.6	100	1,819 ± 85.6	2,400 ± 94.3
	200	22 ± 4.9	73 ± 12.0	200	2,077 ± 130.4	2,148 ± 62.1
	600	17 ± 2.6	63 ± 5.6	600	1,878 ± 17.6	1,312 ± 114.4
	1,000	27 ± 2.0	47 ± 5.3	1,000	1,647 ± 60.1	1,169 ± 164.0
2-NF	3.0	200 ± 10.5				
2-AA	0.5		470 ± 38.4		NA[a]	NA[a]
CSC	0	24 ± 7.6	41 ± 9.0			
	10	18 ± 0.0	102 ± 0.0			
	30	—	207 ± 0.0			
	50	—	191 ± 0.0			
2-NF	3.0	202 ± 24.9				
2-AA	0.5		636 ± 6.0		NA[a]	NA[a]
Coke oven	0	26 ± 6.0	46 ± 4.0			
	20	34 ± 1.7	216 ± 20.7			
	60	28 ± 4.4	356 ± 29.6			
	100	25 ± 4.2	367 ± 21.1			
	200	26 ± 1.5	268 ± 6.0			
	600	24 ± 11.3	105 ± 3.0			
	1,000	14 ± 2.5	58 ± 5.3			
2-NF	3.0	204 ± 7.0		3.0	204 ± 7.0	
2-AA	0.5		512 ± 52.2	0.5		512 ± 52.2
Roofing tar	0	37 ± 8.7	45 ± 10.8	0	37 ± 8.7	45 ± 10.8
	20	21 ± 3.1	75 ± 6.4	20	1,494 ± 99.2	1,358 ± 131.4
	60	20 ± 4.4	90 ± 12.1	60	2,072 ± 79.0	2,801 ± 44.5
	100	19 ± 0.6	95 ± 7.5	100	1,952 ± 50.4	2,780 ± 85.8
	200	17 ± 8.5	69 ± 6.7	200	1,561 ± 111.8	2,791 ± 120.2
	600	14 ± 1.5	18 ± 2.5	600	834 ± 54.4	2,173 ± 154.1
	1,000	6 ± 2.5	10 ± 5.3			

*When possible, these values represent a mean of triplicate plates.
[a]Fraction not available as described in text.

fraction of all four emissions was negative in the absence of S9, further substantiating that this fraction contained purified PNAs. This PNA1 fraction, however, accounted for only a minor portion of the total mutagenicity of the CSC (0.1%) and diesel organics (0.2%). Although the PNA1 fraction made a greater contribution to the mutagenicity of the roofing tar (4.9%) and coke oven main (7.9%), this fraction was less important than the more polar fractions.

Unsubstituted PNAs were also detected in the NPN fraction (Williams et al, unpublished results), which generally contains non-mutagenic aliphatic hydrocarbons.

TABLE IIB. (Continued)

Dose (μg)	PNA3 Fraction rev/plate mean ± SD −S9	PNA3 Fraction rev/plate mean ± SD +S9	Dose (μg)	PNA4 Fraction rev/plate mean ± SD −S9	PNA4 Fraction rev/plate mean ± SD +S9
3.0	204 ± 7.0		3.0	204 ± 7.0	
0.5		512 ± 52.2	0.5		512 ± 52.2
0	37 ± 8.7	45 ± 10.8	0	37 ± 8.7	45 ± 10.8
20	104 ± 6.1	143 ± 14.1	20	46 ± 5.5	59 ± 5.3
60	209 ± 16.2	425 ± 6.7	60	58 ± 3.2	84 ± 6.0
100	283 ± 20.5	797 ± 27.5	100	63 ± 5.6	105 ± 27.4
200	372 ± 13.3	1,614 ± 64.8	200	97 ± 8.4	165 ± 6.0
600	503 ± 14.8	2,018 ± 155.6	600	157 ± 5.5	315 ± 28.3
			1,000	190 ± 4.9	441 ± 36.7
3.0	200 ± 10.5		3.0	200 ± 10.5	
0.5		470 ± 38.4	0.5		470 ± 38.4
0	24 ± 7.6	41 ± 9.0	0	24 ± 7.6	41 ± 9.0
10	30 ± 1.4	51 ± 0.0	10	32 ± 4.7	64 ± 9.4
30	38 ± 13.4	63 ± 0.0	30	50 ± 9.5	62 ± 6.0
50	60 ± 0.0	69 ± 0.0	50	61 ± 10.5	82 ± 8.5
100	65 ± 5.7	105 ± 0.0	100	109 ± 6.6	134 ± 9.5
			300	158 ± 6.6	386 ± 26.7
			500	—	519 ± 0.0
3.0	202 ± 24.9		3.0	202 ± 24.9	
0.5		636 ± 6.0	0.5		636 ± 6.0
0	26 ± 6.0	46 ± 4.0	0	26 ± 6.0	46 ± 4.0
20	36 ± 2.9	211 ± 2.3	10	114 ± 12.2	213 ± 13.0
60	37 ± 5.0	307 ± 19.7	30	185 ± 16.8	583 ± 47.5
100	39 ± 3.6	350 ± 27.1	50	220 ± 9.6	807 ± 11.1
200	41 ± 3.5	365 ± 15.5	100	295 ± 11.5	1,169 ± 38.4
600	41 ± 4.0	304 ± 44.2	300	405 ± 9.0	1,323 ± 60.1
1,000	43 ± 15.7	235 ± 8.6	500	298 ± 0.0	1,285 ± 58.7
3.0	211 ± 9.5		3.0	204 ± 7.0	
0.5		512 ± 52.2	0.5		512 ± 52.2
0	37 ± 8.7	45 ± 10.8	0	37 ± 8.7	45 ± 10.8
10	270 ± 7.8	775 ± 0.0	10	46 ± 9.7	93 ± 10.4
30	—	1,468 ± 0.0	30	84 ± 7.0	285 ± 17.9
50	381 ± 0.0	1,765 ± 0.0	50	100 ± 10.5	428 ± 9.5
			100	122 ± 19.3	651 ± 14.0
			300	180 ± 9.2	677 ± 44.3

The NPN fraction of all four emissions reported here was mutagenic only in the presence of S9 and accounted for 1.4–6.6% of the mutagenicity of these samples. If we assume that these two fractions, PNA1 and NPN, contain all of the unsubstituted PNAs in each of the four samples, then as much as 14.5% of the coke oven and 8.8% of the roofing tar samples' mutagenicity is accounted for by the PNA-containing fractions. In the cases of the diesel and CSC, only 1.4% and 2.2%, respectively, of the total mutagenicity can be attributed to unsubstituted PNAs. This is consistent with previous studies of diesel particulate emissions [Huisingh et al, 1978] and CSC

TABLE IIC. Mutagenicity of Organic Fractions From Four Environmental Samples Using *Salmonella typhimurium* Strain TA98: PN, CI Fractions*

Sample and controls	Dose (μg)	PN Fraction rev/plate mean ± SD −S9	PN Fraction rev/plate mean ± SD +S9	Dose (μg)	CI Fraction rev/plate mean ± SD −S9	CI Fraction rev/plate mean ± SD +S9
2-NF	3.0	118 ± 20.0		3.0	280 ± 14.6	
2-AA	0.5		326 ± 17.0	0.5		389 ± 27.3
Diesel	0	18 ± 3.2	22 ± 3.6	0	34 ± 11.5	35 ± 11.4
	20	213 ± 13.6	59 ± 23.1	20	173 ± 29.7	85 ± 5.0
	60	593 ± 35.2	303 ± 2.6	60	409 ± 5.0	236 ± 13.1
	100	948 ± 177.9	543 ± 43.0	100	758 ± 48.2	373 ± 17.1
	200	1,719 ± 95.8	1,478 ± 102.2	200	1,326 ± 45.0	749 ± 39.3
	600	1,974 ± 18.3	2,435 ± 124.3	600	1,707 ± 77.9	1,715 ± 74.7
	1,000	1,526 ± 184.5	2,301 ± 62.0	1,000	1,613 ± 33.9	2,159 ± 95.7
2-NF	3.0	118 ± 20.0		3.0	280 ± 14.6	
2-AA	0.5		326 ± 17.0	0.5		389 ± 27.3
CSC	0	18 ± 3.2	22 ± 3.6	0	34 ± 11.5	35 ± 11.4
	20	19 ± 5.3	25 ± 4.6	20	26 ± 4.6	41 ± 1.7
	60	13 ± 2.3	28 ± 11.8	60	33 ± 5.8	57 ± 9.8
	100	19 ± 7.5	27 ± 8.7	100	30 ± 7.4	52 ± 8.0
	200	17 ± 1.5	26 ± 5.9	200	35 ± 3.1	62 ± 7.9
	600	21 ± 6.1	38 ± 9.6	600	24 ± 6.9	96 ± 15.9
	1,000	13 ± 1.7	33 ± 4.0	1,000	27 ± 2.6	97 ± 17.3
2-NF	3.0	118 ± 20.0		3.0	280 ± 14.6	
2-AA	0.5		326 ± 17.0	0.5		389 ± 27.3
Coke oven	0	18 ± 3.2	22 ± 3.6	0	34 ± 11.5	35 ± 11.4
	20	21 ± 5.0	94 ± 6.1	20	42 ± 3.8	160 ± 20.8
	60	18 ± 6.8	323 ± 27.4	60	44 ± 4.0	356 ± 18.9
	100	28 ± 9.5	426 ± 10.6	100	39 ± 4.9	467 ± 11.1
	200	29 ± 9.1	534 ± 34.5	200	36 ± 3.5	620 ± 18.9
	600	17 ± 4.0	436 ± 41.7	600	43 ± 10.4	727 ± 69.1
	1,000	12 ± 6.7	165 ± 23.3	1,000	42 ± 7.8	740 ± 29.1
2-NF	3.0	118 ± 20.0		3.0	280 ± 14.6	
2-AA	0.5		326 ± 17.0	0.5		389 ± 27.3
Roofing tar	0	18 ± 3.2	22 ± 3.6	0	34 ± 11.5	35 ± 11.4
	20	72 ± 9.6	55 ± 9.9	20	3,265 ± 79.2	2,092 ± 115.4
	60	169 ± 12.2	139 ± 9.0	60	3,400 ± 30.0	3,165 ± 129.3
	100	184 ± 14.0	203 ± 10.4	100	3,380 ± 55.7	3,134 ± 67.6
	200	159 ± 20.1	407 ± 30.1	200	3,375 ± 109.7	1,886 ± 47.8
	600	299 ± 78.6	180 ± 70.9	600	2,373 ± 100.0	1,187 ± 192.1
	1,000	113 ± 157.5	35 ± 14.4	1,000	386 ± 69.5	326 ± 36.5

*When possible, these values represent a mean of triplicate plates.

[Hutton and Hackney, 1975; DeMarini, 1981] using somewhat different fractionation procedures. Without carcinogenesis data on each of these fractions, it is difficult to estimate the contribution of these PNAs to the total carcinogenicity of the samples.

Although the PNA fraction of each of these emissions accounted for only 1.4–14.5% of the mutagenicity in *Salmonella*, there is evidence that neutral PNA fractions from similar emissions account for most of the tumorigenicity in mouse skin [Jacob, 1983]. In at least two complex mixtures, CSC [DeMarini, 1983] and coal oil [Holland et al, 1984], where mouse skin tumor activity has been compared to *Salmonella* mutagenicity for the same fractions, the fractions accounting for the most mutagenic-

TABLE III. Distribution of Mutagenic Activity in Diesel Automotive Emissions

Fraction	% Mass	Specific mutagenic activity (rev/μg)[a] −S9	Specific mutagenic activity (rev/μg)[a] +S9	Weighted mutagenic activity (rev/μg) −S9	Weighted mutagenic activity (rev/μg) +S9	Distribution of mutagenic activity (%) −S9	Distribution of mutagenic activity (%) +S9
Acid	3.6	6.1 (5.7–6.5)	3.0 (2.9–3.1)	0.2	0.1	9.6	4.1
Base	1.0	10.7 (7.9–14.3)	10.1 (6.1–16.9)	0.1	0.1	4.7	3.8
NPN	75.1	Neg	0.05 (0.04–0.05)	Neg	0.04	Neg	1.4
PNA1	1.1	Neg	0.4 (0.3–0.5)	Neg	0.004	Neg	0.2
PNA2	1.4	12.8 (12.6–13.0)	25.9 (25.8–26.0)	0.2	0.4	7.8	13.6
PNA3	0.7	1.9 (1.4–2.5)	29.8 (26.7–33.3)	0.01	0.2	0.6	7.8
PNA4	1.3	0.3 (0.1–1.1)	0.5 (0.4–0.7)	0.004	0.01	0.2	0.2
PN	7.7	13.6 (13.2–14.0)	18.2 (17.6–18.8)	1.1	1.4	45.5	52.5
CI	8.1	9.0 (8.7–9.3)	5.4 (4.9–6.0)	0.7	0.4	31.7	16.4
Total fractionated				2.3	2.6		
Unfractionated (NEAT)		2.1 (1.9–2.4)	1.4 (1.2–1.7)				
% Recovery	86.8			109.5	185.7		

[a]Non-linear model slope as determined by Stead et al [1980] (95% confidence limits).

TABLE IV. Distribution of Mutagenic Activity in Cigarette Smoke Condensate

Fraction	% Mass	Specific mutagenic activity (rev/μg)[a] −S9	Specific mutagenic activity (rev/μg)[a] +S9	Weighted mutagenic activity (rev/μg) −S9	Weighted mutagenic activity (rev/μg) +S9	Distribution of mutagenic activity (%) −S9	Distribution of mutagenic activity (%) +S9
Acid	1.0	Neg	0.2 (0.1–0.2)	Neg	0.002	Neg	0.7
Base	12.2	Neg	1.6 (1.1–2.4)	Neg	0.2	Neg	66.1
NPN	0.6	Neg	1.1 (0.4–3.1)	Neg	0.01	Neg	2.2
PNA1	0.01	Neg	3.5[b]	Neg	3.5^{-4}	Neg	0.1
PNA2	—	—	—	—	—	—	—
PNA3	0.1	2.1 (0.04–112.6)	0.6 (0.1–2.5)	0.001	4.2^{-4}	13.0	0.1
PNA4	0.5	1.9 (0.7–5.5)	3.2 (1.3–7.6)	0.01	0.02	87.1	5.6
PN	11.6	Neg	Neg	Neg	Neg	Neg	Neg
CI	74.0	Neg	0.1 (0.1–0.3)	Neg	0.1	Neg	25.1
Total fractionated				0.011	0.3		
Unfractionated (NEAT)		Neg	1.1 (0.5–2.4)				
% Recovery	47.6				27.3		

[a]Non-linear model slope as determined by Stead et al [1980] (95% confidence limits).
[b]Slope calculated from linear regression analysis of the data owing to insufficient mass of this fraction available for adequate data to determine model slope.

ity (eg, basic fraction of CSC) were not the same fractions that accounted for the most skin tumorigenicity. The mouse skin tumor assay has been widely used to detect the carcinogenicity of both individual PNAs as well as complex mixtures of polycyclic organic matter. In order to better understand the apparent discrepancy in response between these two assays, more data is needed comparing the activity of both fractions and individual compounds in the mouse skin tumor assay and the *Salmonella typhimurium* mutagenicity assay.

TABLE V. Distribution of Mutagenic Activity in Coke Oven Emissions

Fraction	% Mass	Specific mutagenic activity (rev/μg)[a] −S9	Specific mutagenic activity (rev/μg)[a] +S9	Weighted mutagenic activity (rev/μg) −S9	Weighted mutagenic activity (rev/μg) +S9	Distribution of mutagenic activity (%) −S9	Distribution of mutagenic activity (%) +S9
Acid	0.3	Neg	1.8 (0.2–13.4)	Neg	0.01	Neg	0.1
Base	3.9	Neg	44.2 (43.8–44.5)	Neg	1.7	Neg	37.1
NPN	13.9	Neg	2.2 (2.0–2.3)	Neg	0.3	Neg	6.6
PNA1	9.0	Neg	4.1 (3.9–4.3)	Neg	0.4	Neg	7.9
PNA2	—	—	—	—	—	—	—
PNA3	7.4	0.1 (0.1–0.2)	3.4 (3.1–3.6)	0.01	0.3	18.1	5.4
PNA4	1.4	2.4 (2.0–2.7)	12.2 (11.8–12.6)	0.03	0.2	82.0	3.7
PN	6.5	Neg	5.9 (5.6–6.1)	Neg	0.4	Neg	8.2
CI	57.6	Neg	2.5 (2.3–2.6)	Neg	1.4	Neg	31.0
Total fractionated				0.04	4.7		
Unfractionated (NEAT)		Neg	3.5 (3.4–3.7)				
% Recovery	83.6				134.3		

[a]Non-linear model slope as determined by Stead et al [1980] (95% confidence limits).

TABLE VI. Distribution of Mutagenic Activity in Roofing Tar Emissions

Fraction	% Mass	Specific mutagenic activity (rev/μg)[a] −S9	Specific mutagenic activity (rev/μg)[a] +S9	Weighted mutagenic activity (rev/μg) −S9	Weighted mutagenic activity (rev/μg) +S9	Distribution of mutagenic activity (%) −S9	Distribution of mutagenic activity (%) +S9
Acid	2.3	Neg	2.5 (1.5–3.9)	Neg	0.1	Neg	1.1
Base	2.9	Neg	10.5 (10.3–10.7)	Neg	0.3	Neg	5.9
NPN	40.4	Neg	0.5 (0.5–0.6)	Neg	0.2	Neg	3.9
PNA1	31.8	Neg	0.8 (0.6–0.9)	Neg	0.3	Neg	4.9
PNA2	1.2	24.5 (24.2–24.8)	33.8 (33.6–34.0)	0.3	0.4	6.9	7.9
PNA3	0.02	6.6[b]	35.4 (34.2–36.6)	0.001	0.01	0.03	0.1
PNA4	0.3	1.0 (0.6–1.7)	16.5 (13.9–19.7)	0.003	0.05	0.1	1.0
PN	13.1	1.5 (1.5–1.6)	6.8 (6.3–7.3)	0.2	0.9	4.6	17.3
CI	8.0	47.4 (47.3–47.6)	37.4 (37.3–37.5)	3.8	3.0	88.5	57.9
Total unfractionated				4.3	5.3		
Unfractionated (NEAT)		Neg	6.0 (0.5–78.1)				
% Recovery	83.6				88.3		

[a]Non-linear model slope as determined by Stead et al [1980] (95% confidence limits).
[b]Slope calculated from linear regression analysis of the data owing to insufficient mass of this fraction available for adequate data to determine model slope.

The NO_2-PNAs, many of which are mutagenic in the absence of S9 [Mermelstein et al, 1981], were found in the PNA2 and PNA3 fractions (Williams et al, unpublished results). The PNA2 fraction of the diesel and roofing tar samples were highly mutagenic in the absence of S9. Only the diesel sample, however, contained detectable quantities of NO_2-PNAs. The Oldsmobile diesel sample reported here contained 107 ppm 1-nitropyrene and lesser amounts of other NO_2-PNAs [Nishioka et al, 1982]. The PNA2 fraction accounted for 7.8% of the diesel mutagenicity in the absence of S9 and 13.6% in the presence of S9, while the PNA3 fraction accounted for 0.6% and 7.8%, respectively.

The two most polar fractions, PN and CI, together accounted for most of the mutagenicity (69% and 77%) in the presence and absence of S9, respectively, for the diesel sample. Although chemical characterization of these fractions shows the presence of oxygenated polycyclic aromatic compounds (eg, quinones, diones, and nitro-oxygenated compounds), the specific compounds responsible for the mutagenicity have not been identified (Williams et al, unpublished results) [Schuetzle, 1983]. These two polar fractions also account for major portions of the mutagenicity of the roofing tar (74%), coke oven (39%), and CSC (25%) samples. If the mutagenicity of the polar contaminants of the PNAs (eg, PNA2–4) is added to the mutagenicity of the PN and CI fractions, most of the mutagenicity of the diesel sample (90%) and roofing tar sample (83%) can be accounted for in these polar neutral fractions and lesser amounts in the coke oven (48%) and CSC (31%).

The other fraction that makes a major contribution to the mutagenicity of the coke oven main and CSC is the BASE fraction, which is mutagenic only in the presence of S9. This fraction accounts for 37% of the mutagenicity of the coke oven main and has been shown to contain mutagenic aromatic amines and nitrogen heterocycles (Williams et al, unpublished results; Nishioka and Petersen, personal communication). This finding suggests that such nitrogen-containing compounds may be more important than the unsubstituted PNAs in contributing to the mutagenicity of coke oven emissions. This is also consistent with reports that coke oven topside workers have an excess of kidney and possibly bladder cancer [Redmond et al, 1976; Radford, 1976] in addition to the well-documented excess of lung cancer [Lloyd, 1971, 1980; Teplitz et al, 1980].

Although the CSC sample also had a very high percentage of the mutagenic activity in the BASE fraction (66%) (which is in agreement with other studies of CSC [Hutton and Hackney, 1975]), the chemical analysis indicated significant differences between the constituents of the CSC and coke oven emissions (Williams et al, unpublished results). This is not surprising since coke oven emissions result from the reductive distillation of coal, a fossilized carbon source, and the cigarette emissions result from the oxidative combustion of a vegetative carbon source containing more cellulose and amino acid components. Sugimura [1982] has reported the presence of highly mutagenic heterocyclic amines (eg, 2-amino-9H-pyrido[2,3,-6]indole) in CSC, and Mizusaki et al [1977] proposed that these mutagens resulted from the pyrolysis of amino acids.

The ACID fraction, although making a major contribution to the mutagenicity of urban air particulate extracts [Lewtas et al, 1982; Alfheim et al, 1983], makes a very minor contribution (0.1–1.1%) to the CSC, coke oven, and roofing tar samples and contributes only 4.1–9.6% of the mutagenicity of the diesel sample. This fraction also contains highly polar compounds (eg, phenolic and carboxylated organics) that are acidic.

It is clear that the specific mutagens in these different sources are not identical, however, they all cause frameshift mutations and appear to be compounds that could be classified as polycyclic organic matter (POM). Chemical characterization suggests that in addition to NO_2-PNAs found in the slightly and moderately polar neutrals (PNA1 and PNA2), hydroxylated and carboxylated polycyclic organics are found in the organic ACID fraction; aromatic amines and nitrogen heterocycles are found in the organic BASE fraction; and highly oxygenated quinones, diones, and nitro-oxygenated compounds are found in the highly polar neutral fractions (PN and CI).

The similarity between these four sources, therefore, resides in the general polycyclic organic content and mechanism of frameshift mutations rather than in the specific mutagens present in these emissions. It is important to note that this study is based on only one emission sample of each type. It is likely that there will be some variation in the distribution of mutagens in the fractions from emission samples collected from the same source but at different times.

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